

Replace the paragraph beginning at page 25, line 2, with the following rewritten paragraph:

A2

--*Figure 5* is a schematic representation of the fourteen fragments (Fragments A-Fragment N) assembled to construct pXF8.61 (coding and non-coding strands are SEQ ID NOs:107-120 and 121-134, respectively).--

Replace the paragraph beginning at page 25, line 5, with the following rewritten paragraph:

A3

--*Figure 7* depicts the nucleotide sequence and the corresponding amino acid sequence of the LE B-domain-deleted-Factor VIII (FVIII) insert contained in pAM1-1 (SEQ ID NOs:1 and 3, respectively).--

Replace the paragraph beginning at page 25, line 7, with the following rewritten paragraph:

A4

--*Figure 8* is a schematic representation of the fragments assembled to construct pXF8.186 (coding and non-coding strands are SEQ ID NOs:135 and 136, respectively).--

Replace the paragraph beginning at page 25, line 8, with the following rewritten paragraph:

A5

--*Figure 9* depicts the nucleotide sequence and the corresponding amino acid sequence of the 5Arg B-domain-deleted-FVIII insert (SEQ ID NOs:2 and 4, respectively).--

Replace the paragraph beginning at page 45, line 23, with the following rewritten paragraph:

A6

--Human Factor VIII expression plasmids, plasmids pXF8.186 (Figure 3), pXF8.61 (Figure 4), pXF8.38 (Fig. 11) and pXF8.224 (Fig. 13) are described below. The hFVIII expression construct plasmid pXF8.186, was developed based on detailed optimization studies which resulted in high level expression of a functional hFVIII. Given the extremely large size of the hFVIII gene and the need to transfer the entire coding region into cells, cDNA expression

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CONT.

plasmids were developed for the production of stably transfected clonal cell strains. It has proven difficult to achieve high level expression of hFVIII using the wild-type 9 kb cDNA. Three potential reasons for the poor expression are as follows. First, the wild-type cDNA encodes the 909 aa, heavily glycosylated B-domain which is transiently attached to the heavy chain and has no known function (Figure 1). Removal of the region encoding the B-domain from hFVIII expression constructs leads to greatly improved expression of a functional protein. Analysis of hFVIII derivatives lacking the B-domain has demonstrated that hFVIII function is not adversely affected and that such molecules have biochemical, immunologic, and in vivo functional properties which are very similar to the wild-type protein. Two different BDD hFVIII expression constructs have been developed, which encode proteins with different amino acid sequences flanking the deletion. Plasmid pXF8.186 contains a complete deletion of the B-domain (amino acids 741-1648 of the wild-type mature protein sequence), with the sequence Arg-Arg-Arg-Arg (RRRR; SEQ ID NO:137) inserted at the heavy chain-light chain junction (Figure 1). This results in a string of five consecutive arginine residues (RRRRR or 5R; SEQ ID NO:138) at the heavy chain-light chain junction, which comprises a recognition site for an intracellular protease of the PACE/furin class, and was predicted to promote cleavage to produce the correct heavy and light chains. Plasmid pXF8.61 also contains a complete deletion of the B-domain with a synthetic XhoI site at the junction. This linker results in the presence of the dipeptide sequence Leu-Glu (LE) at the heavy chain-light chain junction in the two forms of BDD hFVIII, the expressed proteins are referred to herein as 5R and LE BDD hFVIII.--

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